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X marks the spot: PRDM9 rescues hybrid sterility by finding hidden treasure in the genome

Maciej J. Zelazowski and **Francesca Cole**

Department of Epigenetics and Molecular Carcinogenesis, University of Texas MD Anderson Cancer Center, Smithville, Texas, USA

Three papers, Smagulova, Brick, et al.[1], Patel, et al.[2], and Davies, Hatton et al.[3] provide compelling evidence for how PRDM9 binds to meiotic hotspots leading to hotspot erosion and speciation.

In sexually reproducing organisms, meiosis halves the chromosome complement to generate haploid gametes. To do so, parental chromosomes (homologs) must become paired and synapsed (Fig1). In mammals and many organisms, this synapsis is achieved through meiotic recombination. A unique feature of meiosis is that the partner for recombination is the homolog and not the sister chromatid. Recombination is initiated by programmed DNA double-strand breaks (DSBs) that are resected to generate 3′ single-stranded tails that are bound by recombinases RAD51 and DMC1 to engage in homology search. As DSB repair ensues, pairing is gradually reinforced until homologs are fully synapsed.

In most mammals, the meiosis-specific enzyme PRDM9 (PR domain containing 9) determines DSB distribution [4]. PRDM9 contains a tandem array of zinc fingers (ZnFs) that recognize motifs within the genome and, upon binding, deposits histone H3 lysine 4 trimethyl marks (H3K4me3) at neighboring nucleosomes. It is thought that the DSB machinery is then recruited to initiate recombination. As a consequence of PRDM9 action, DSBs are clustered at specific, discrete sites in the genome, referred to as hotspots.

And here be dragons: if a hotspot lies in a region where homologs differ in their PRDM9 binding affinities (Fig2), the homolog with higher affinity receives more DSBs and is hence, “hotter”. DSBs are then repaired by synthesis from the uncut “colder” homolog [4]. As a result, sequences on the cut homolog are frequently converted to those of the uncut homolog (gene conversion). In theory, gene conversion in favor of “colder” sequences, repeated over thousands of generations, would erode PRDM9 binding sites (Fig3). Although erosion should ultimately, extinguish hotspots, they persist - a dilemma termed the “hotspot paradox”[5]. One proposed solution is that rapid evolution of the *Prdm9* ZnF array can alter PRDM9 binding specificity and turn back the erosion clock.

PRDM9 also plays a role in speciation, which has been difficult to reconcile with its aforementioned function in meiotic DSB distribution. Specific combinations of *Prdm9*

*To whom correspondence should be addressed: FCole@MDAnderson.org.

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alleles in two subspecies of mice render the male offspring infertile, referred to as hybrid sterility [6]. Hybrid sterile spermatocytes frequently display asynapsis (lack of pairing) or nonhomologous synapsis (mispairing) of homologs (Fig1) leading to apoptosis and, thereby, compromised fertility [7].

To investigate how different versions of PRDM9 interact to regulate meiotic recombination, Smagulova, Brick et al.[1] generated high-resolution DSB maps of inbred mouse strains and their F1 hybrids using DMC1-ChIP sequencing [8]. By comparing DSB maps among the 6 different inbred strains, they found it was difficult to predict the impact of altered PRDM9 ZnF arrays on hotspot distribution. While some alleles shared only 1% of hotspots with others, a few hotspots did overlap between alleles. However, the extent of hotspot similarity could not be explained simply by comparing ZnF identities between alleles. For example, the ZnF arrays of $PRDM9^{PWD}$ and $PRDM9^{MOL}$ are much more similar to each other than those of $PRDM9^{PWD}$ and $PRDM9^{CAST}$ despite sharing roughly the same number of hotspots with each other (13-14% and 11-12%, respectively). As such, differences between PRDM9 alleles are far from quantitative, and subtle changes can dramatically alter the recombination landscape.

To gain more insight into how PRDM9 recognizes and binds motifs, Patel, et al. [2] co-crystallized ZnF8-12 of the human $PRDM9^{A}$ protein in association with a known hotspot motif. They found that ZnF8-11 contact DNA within the major groove, with most ZnF helices forming hydrogen bond contacts with four adjacent bases. Intriguingly, most of the variable base pairs within the predicted consensus sequence also have extensive hydrogen bonding with the ZnF array. These subsidiary bonds are not important for sequence recognition, but potentially stabilize the interaction if they can form. As such, they appear to compensate for alteration in the conserved consensus sequence, suggesting that PRDM9 can opportunistically associate with less ideal binding motifs.

By determining the in vitro hotspot binding affinities of purified ZnF8-12 alleles, they found that, similar to the genomic studies, subtle protein sequence differences could dramatically alter DNA binding specificity. For example, although the ZnF array of two human PRDM9 alleles, A and C, differ by only a single amino acid, the binding affinity of $PRDM9^{C}$ for C-specific motifs was tenfold higher than that of $PRDM9^{A}$, which is consistent with the partial dominance of the $PRDM9^{C}$ allele in a human genome-wide DSB map [9].

To determine how PRDM9 alleles interact with one another, Smagulova, Brick et al. [1] generated F1 hybrids between each of their inbred mouse strains. Consistent with previous reports [10,11], they observed many novel hotspots in F1 hybrids as compared to the inbred strains. Intriguingly, novel hotspots show extreme asymmetry in their DMC1 signal, forming exclusively on only one of the two parental chromosomes. The DNA sequence motifs associated with these novel hotspots suggest that PRDM9 alleles are specifically binding to the non-self chromosomes.

The simplest interpretation of these findings is that hotspots on non-self chromosomes have PRDM9-binding motifs that have never been exposed to the cognate PRDM9 ZnF array that recognizes them in the F1 hybrid (Fig3). As such, they have been maintained in a pristine

state, whereas any corresponding hotspot on the self-chromosome would have been subjected to hotspot erosion (Fig2). Consistent with this interpretation, most novel hotspots contained centrally-located polymorphisms that are predicted to improve PRDM9 binding.

To definitively assess whether parental bias is a consequence of hotspot erosion, they queried how PRDM9 acts on “non-self” versus “self” X chromosomes using reciprocal crosses. As predicted by the model, PRDM9 preferentially recognizes the “non-self” chromosome in each orientation. These results provide substantial insight into how PRDM9 has specifically altered sequences at hotspots over time, and offer compelling evidence for hotspot erosion.

Davies, Hatton et al. [3] tested the proposed solution to the hotspot paradox: Would introduction of novel allele of PRDM9 reverse hybrid sterility by counteracting the deleterious effects of hotspot erosion? To do so, they humanized the mouse C57BL/6 *Prdm9* allele by replacing the ZnF array sequence with that encoded by a human *Prdm9*. Satisfyingly, when crossed in the hybrid sterile configuration, the presence of the human allele completely rescued fertility (Fig3). They then investigated both the cause of hybrid sterility and how a new *Prdm9* allele may rescue it.

DMC1-ChIP sequencing revealed that the hybrid sterile mice show asymmetric DMC1 signal between parental homologs. By contrast, addition of humanized *Prdm9* led to symmetric DMC1 signal. Intriguingly, when they analyzed a direct consequence of PRDM9 binding, the deposition of H3K4me3, at hotspots, they found that DMC1 signal intensity was highest at hotspots with asymmetric loading of H3K4me3. Increased DMC1 signal could reflect increased DSBs or a longer lifespan of the DMC1 nucleoprotein filament. To discriminate between these two possibilities, the authors compared PRDM9 binding and DMC1 signal at hotspots located in regions that are not shared between homologs, such as the heteromorphic X chromosome. These regions cannot pair and DSB repair is delayed [7]. DMC1 signal strength was five-fold higher in these regions than that at symmetric hotspots, despite similar levels of H3K4me3. The authors conclude that elevated DMC1 signal, when disconnected from PRDM9 binding, could be a hallmark of prolonged DSB repair.

They also observed that PRDM9 acts in *trans* to influence the activity of the other PRDM9 protein in F1 hybrids. A simple interpretation of this finding is that PRDM9 multimerizes to associate with its binding motifs [12]. If so, alteration of PRDM9 binding in *trans* would be expected to change not only the DMC1 signal, but also the level of PRDM9-dependent H3K4me3. Since this was not the case, the *trans* effect influences events downstream of PRDM9 binding. Taken together, these findings suggest an additional role for PRDM9 in promoting efficient pairing of homologs and subsequent DSB repair through symmetric association between homologs.

Spermatocytes from hybrid sterile mice display high levels of unpaired homologs and asynapsis, which is the proposed cause for their death and the infertility (Fig1). Different chromosomes show varying, but reproducible levels of asynapsis in hybrid sterile spermatocytes [7]. The authors compared the published asynapsis levels between chromosomes in hybrid sterile mice to the level of PRDM9 binding asymmetry between

chromosomes. Consistent with a pivotal role for PRDM9 in homolog pairing, they found that chromosome regions that experience high levels of asynapsis correlate well with high levels of PRDM9 binding asymmetry.

The implication of these findings is that symmetric PRDM9 binding promotes homolog pairing and subsequent DSB repair. In the event that too many sites are asymmetrically bound, homologous pairing is compromised, leading to asynapsis and, in the extreme case, hybrid sterility. Introduction of a new PRDM9 can reverse sterility by recognizing novel motifs that have not experienced hotspot erosion and are thus symmetrically associated with PRDM9 between homologs (Fig3).

How could symmetric PRDM9 binding promote homolog pairing? The authors propose a model in which PRDM9 directly aides in homology search. PRDM9 deposits H3K4me3 across approximately 1kb at hotspots, and previous work estimates that PRDM9 binds and modifies ∼5000 hotspots within a spermatocyte [13]. If we make the assumption that homology search is limited to the regions modified by PRDM9, than this would reduce the homology search area by three orders of magnitude.

At face value, this seems like a dangerous situation, because a substantial number of homologous regions could be excluded from the search area. However, experimental evidence from yeast demonstrates that successful interhomolog interactions along a chromosome can reinforce allelic recombination, even between highly divergent sequences [14]. Thus, only a portion of hotspots need have both homologs located within the search area to substantially increase the probability of synapsis along a chromosome.

Reducing asymmetric binding in hybrid sterile situations should reduce the extent of asynapsis. Consistent with this model, increased dosage of PRDM9, which is expected to increase binding at eroded sites, also reduces the severity of hybrid sterility [15]. Importantly, loss of PRDM9 renders male and female mice sterile despite efficient DSB formation [11,16]. The cytological phenotype is reminiscent of hybrid sterile males with a high frequency of asynapsed homologs and nonhomologous synapsis. DSBs in mice lacking PRDM9 are almost entirely symmetric, and occur at sites associated with PRDM9-independent H3K4me3. In this context, symmetry of DSB formation alone is not sufficient to fully pair homologs, which suggests that PRDM9 plays a critical role in homolog pairing downstream of DSB formation.

If symmetric PRDM9 binding is required for homolog pairing in mice, then how do organisms without PRDM9 successfully navigate meiosis? PRDM9 is mammalian–specific and is not found in birds [17] and other species with similarly large and complex genomes (e.g., [18]). Nor is PRDM9 required in all mammals: the gene dysfunctional in the entire canid lineage [19,20] and at least one fertile human being has a loss of function allele [21]. This suggests that the role PRDM9 plays in mice must be either unnecessary or compensated for in other contexts. Determining these alternative pathways and how they may interplay with PRDM9 will be an intriguing area of further study.

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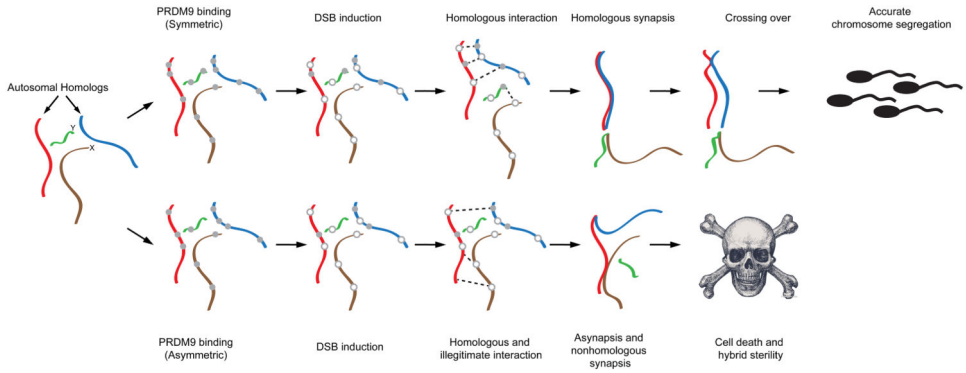

Fig1. Chromosomes pairing and its impact on fertility

Chromosome pairing is initiated by PRDM9 binding at hotspots and depositing H3K4me3 (gray circles); only some sites receive DSBs (white circles). As long as homologs have enough effective interhomolog interactions, pairing is efficient and leads to accurate chromosome segregation and healthy gametes (top). If homologs do not have enough interhomolog interactions, pairing is inefficient and leads to failure of synapsis, cell death, and ultimately, sterility (bottom).

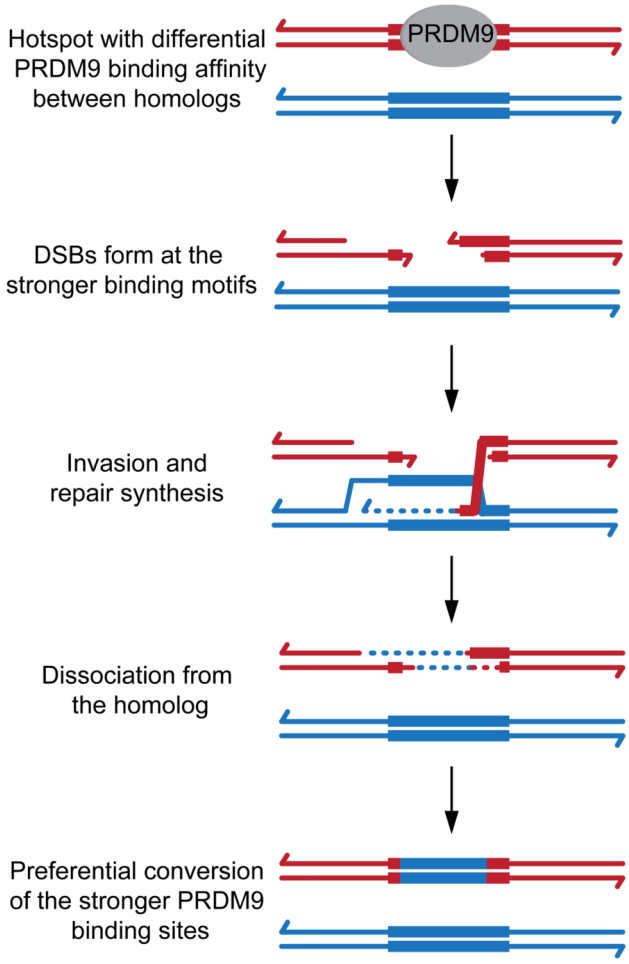

Fig2. Hotspot erosion due to gene conversion

If a hotspot sequence is heterozygotic such that one of the alleles shows higher binding affinity of a PRDM9 allele (red bars), this will cause DSBs to happen more often on the preferred ‘hotter’ allele (red bar). DSB repair by homologous recombination leads to the use of the ‘colder’ (blue bar) allele as a template during repair synthesis. After dissociating and reannealing of the broken strand, a stretch of the ‘hotter’ allele is converted to the ‘colder’ sequence, thus attenuating the PRDM9 binding sequence.

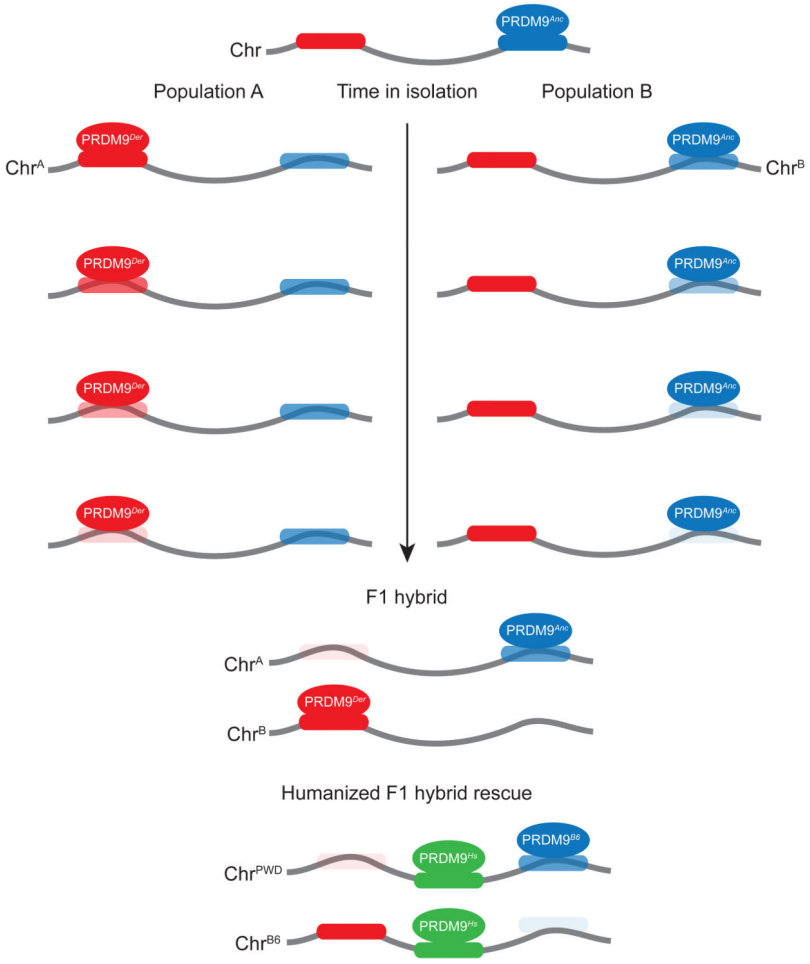

Fig3. Hotspot erosion leads to asymmetric hotspots

In this simple scenario, one ancestral allele of PRDM9 (PRDM9^{Anc}) exists in a population with binding affinity for a particular consensus motif (blue bar). Two populations become isolated and, in one of them, a derived allele of PRDM9 arises (PRDM9^{Der}). PRDM9^{Der} has a different binding consensus motif (red bar) than PRDM9^{Anc}. Throughout generations, PRDM9^{Der}, acting only in population A, will erode its binding motifs on the self-chromosome (Chr^{A}), leaving other sequences unaffected. Analogously, in population B, PRDM9^{Anc} continues to erode its consensus motif on Chr^{B}, ultimately fully attenuating the motif. If animals from those two populations interbreed, generating an F1 hybrid, PRDM9^{Der} will then be able to bind with high affinity to motifs that had not experienced erosion on the non-self chromosome, and vice versa. Hotspots are thus highly asymmetric, as was found in the novel hotspots in F1 hybrids described in the text. The asymmetry can be reversed by introduction of a new allele (e.g., PRDM9^{H}); which can find many high-affinity and symmetric binding motifs (green bars) on both parental chromosomes.